

What is claimed is:

1. An  $\alpha$ -keto acid reductase having the following physicochemical properties:
  - (i) function:
    - 5 reduces  $\alpha$ -keto acid to produce (R)- $\alpha$ -hydroxy acid using reduced  $\beta$ -nicotinamide adenine dinucleotide as the coenzyme; and
    - (ii) substrate specificity:
      - (a) utilizes reduced  $\beta$ -nicotinamide adenine dinucleotide as the coenzyme in the reduction reaction of (i);
      - 10 (b) reducing 2-chlorophenyl glyoxylic acid to produce (R)-2-chloromandelic acid; and
      - (c) reduces 2-chlorophenyl glyoxylic acid but substantially fails to dehydrogenate either of the two optical isomers of 2-chloromandelic acid.
  - 15 2. The  $\alpha$ -keto acid reductase of claim 1, further having the following physicochemical properties:
    - (iii) optimum pH:  
pH 5.0 to 5.5;
    - (iv) optimum temperature:  
20 45 to 55°C; and
    - (v) molecular weight of  
about 35,000 Daltons and about 63,000 Daltons, as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and gel filtration, respectively.
  - 25 3. The  $\alpha$ -keto acid reductase of claim 1, which is produced by a microorganism belonging to the genus *Leuconostoc*.
  4. The  $\alpha$ -keto acid reductase of claim 3, wherein the microorganism belonging to the genus *Leuconostoc* is *Leuconostoc mesenteroides*.
  - 30 5. The  $\alpha$ -keto acid reductase of claim 4, wherein the microorganism belonging to *Leuconostoc mesenteroides* is *Leuconostoc mesenteroides* subsp. *dextranicum*.

6. A polynucleotide encoding a protein, wherein said protein is an enzyme that catalyzes the reduction of  $\alpha$ -keto acids, and wherein said polynucleotide is selected from the group consisting of:

- 5 (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO: 1;
- (b) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO: 2;
- (c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO: 2, wherein one or more amino acids have been substituted, deleted, inserted, and/or added;
- 10 (d) a polynucleotide hybridizing under stringent conditions to a DNA comprising the nucleotide sequence of SEQ ID NO: 1; and
- (e) a polynucleotide encoding an amino acid sequence which exhibits 50% or higher homology to the amino acid sequence of SEQ ID NO: 2.

15 7. A protein encoded by the polynucleotide of claim 6.

8. A recombinant vector wherein the polynucleotide of claim 6 has been inserted.

9. The recombinant vector of claim 8, wherein a polynucleotide encoding a  
20 dehydrogenase catalyzing an oxidation-reduction reaction using  $\beta$ -nicotinamide adenine dinucleotide as the coenzyme has been further inserted.

10. The vector of claim 9, wherein the dehydrogenase is a formate dehydrogenase.  
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11. The vector of claim 10, wherein the formate dehydrogenase is derived from *Mycobacterium vaccae*.

12. The vector of claim 9, wherein the dehydrogenase is a glucose dehydrogenase.  
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13. The recombinant vector of claim 12, wherein the glucose dehydrogenase is derived from *Bacillus subtilis*.

35 14. A transformant comprising any one of the polynucleotides of claim 6 in an expressible manner.

15. A method for producing the protein of claim 7, wherein said method comprises the steps of culturing a transformant comprising any one of the polynucleotides selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO: 1;
- 5 (b) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO: 2;
- (c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO: 2, wherein one or more amino acids have been substituted, deleted, inserted, and/or added;
- 10 (d) a polynucleotide hybridizing under stringent conditions to a DNA comprising the nucleotide sequence of SEQ ID NO: 1; and
- (e) a polynucleotide encoding an amino acid sequence which exhibits 50% or higher homology to the amino acid sequence of SEQ ID NO: 2,  
and collecting the expressed product.

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16. A method for producing the enzyme of claim 1, wherein said method comprises the step of culturing a microorganism belonging to the genus *Leuconostoc*.

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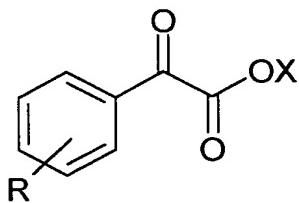
17. The method of claim 16, wherein the microorganism belonging to the genus *Leuconostoc* is *Leuconostoc mesenteroides*.

18. The method of claim 17, wherein the microorganism belonging to *Leuconostoc mesenteroides* is *Leuconostoc mesenteroides* subsp. *dextranicum*.

19. A method for producing an optically active  $\alpha$ -hydroxy acid, wherein said method comprises the following sequential steps:

- (i) reacting
  - (a) the  $\alpha$ -keto acid reductase of claim 1;
  - 5 (b) a protein encoded by a polynucleotide selected from the group consisting of:
    - (1) a polynucleotide comprising the nucleotide sequence of SEQ ID NO: 1;
    - (2) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO: 2;
    - 10 (3) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO: 2, wherein one or more amino acids have been substituted, deleted, inserted, and/or added;
    - (4) a polynucleotide hybridizing under stringent conditions to a DNA comprising the nucleotide sequence of SEQ ID NO: 1; and
    - 15 (5) a polynucleotide encoding an amino acid sequence which exhibits 50% or higher homology to the amino acid sequence of SEQ ID NO: 2;
    - (c) a microorganism producing said  $\alpha$ -keto reductase or said protein; or
    - (d) a processed product of the microorganism with an  $\alpha$ -keto acid; and
  - 20 (ii) collecting the optically active  $\alpha$ -hydroxy acid produced in step (i).

20. The method of claim 19, wherein the  $\alpha$ -keto acid is a phenylglyoxylic acid derivative of formula (I):



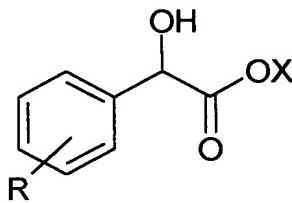
25 formula (I)

wherein:

X is a hydrogen atom, an alkaline metal, or a alkaline earth metal; and

- R indicates one or more substituents at the ortho, meta, or para positions
- 30 selected from the group consisting of a halogen atom, a hydroxyl group, a C<sub>1-3</sub> alkyl group, a C<sub>1-3</sub> alkoxy group, a C<sub>1-3</sub> thioalkyl group, an amino group, a nitro group, a mercapto group, a phenyl group, and a phenoxy group,
- and wherein said method comprises the step of collecting the optically produced active

mandelic acid derivative of formula (II):



formula (II)

5 wherein X and R are as defined in Formula (I).

21. The method of claim 20, wherein the ortho position of the phenylglyoxylic acid derivative is substituted.

10 22. The method of claim 21, wherein the ortho position of the phenylglyoxylic acid derivative is substituted with a halogen atom.

23. The method of claim 20, wherein the meta position of the phenylglyoxylic acid derivative is substituted.

15 24. The method of claim 23, wherein the meta position of the phenylglyoxylic acid derivative is substituted with a halogen atom.

25. The method of claim 19, wherein the  $\alpha$ -keto acid is 2-chlorophenyl glyoxylic acid and the optically active  $\alpha$ -hydroxy acid is (R)-2-chloromandelic acid.

26. The method of claim 19, wherein the microorganism is a transformant any one of the polynucleotides selected from the group consisting of

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO: 1;  
25 (b) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO: 2;

(c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO: 2, wherein one or more amino acids have been substituted, deleted, inserted, and/or added;

30 (d) a polynucleotide hybridizing under stringent conditions to a DNA comprising the nucleotide sequence of SEQ ID NO: 1; and

(e) a polynucleotide encoding an amino acid sequence which exhibits 50% or higher homology to the amino acid sequence of SEQ ID NO: 2.

27. The method of claim 19, wherein said method further comprises the step of converting oxidized  $\beta$ -nicotinamide adenine dinucleotide to reduced  $\beta$ -nicotinamide adenine dinucleotide.

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28. The method of claim 27, wherein the oxidized  $\beta$ -nicotinamide adenine dinucleotide is converted to reduced  $\beta$ -nicotinamide adenine dinucleotide by the function of an enzyme that catalyzes dehydrogenation using oxidized  $\beta$ -nicotinamide adenine dinucleotide as the coenzyme.

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29. The method of claim 28, wherein the enzyme that catalyzes dehydrogenation using oxidized  $\beta$ -nicotinamide adenine dinucleotide as the coenzyme is formate dehydrogenase and/or glucose dehydrogenase.